

# Scopoletin in Commercial Tobacco Products

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Table 1.—Determination of scopoletin in tobacco

Tobacco product type and brand	Volume (ml) aliquot streaked on paper	Scopoletin ( $\mu$ g) per aliquot	Scopoletin ( $\mu$ g) per 1 g tobacco*	Scopoletin (%) in tobacco
(1)	(2)	(3)	(4)	(5)
<b>Cigars</b>				
1C				
Sample 1	10	18.4	92.0	0.0092
Sample 2	10	17.6	88.0	0.0088
2C				
Sample 1	10	19.3	96.5	0.0097
Sample 2	10	19.1	95.5	0.0096
3C				
Sample 1	10	5.8	29.0	0.0029
Sample 2	10	5.5	27.5	0.0028
4C				
Sample 1	10	7.3	36.5	0.0037
Sample 2	10	7.3	36.5	0.0037
<b>Hand-rolled Cigarette Tobacco</b>				
1HC				
Sample 1	15	26.1	87.0	0.0087
Sample 2	15	27.3	91.0	0.0091
2HC				
Sample 1	15	28.5	95.0	0.0095
Sample 2	15	28.0	93.3	0.0093
<b>Pipe Mixtures</b>				
1PM				
Sample 1	15	23.7	79.0	0.0079
Sample 2	15	23.5	78.3	0.0078
2PM				
Sample 1	15	26.0	86.7	0.0087
Sample 2	15	28.8	96.0	0.0096
3PM				
Sample 1	15	25.3	84.3	0.0084
Sample 2	15	24.4	81.3	0.0081
<b>Pipe Tobaccos</b>				
1P				
Sample 1	15	17.8	59.3	0.0059
Sample 2	15	18.7	62.3	0.0062
2P				
Sample 1	15	13.8	46.0	0.0046
Sample 2	15	14.4	48.0	0.0048
3P				
Sample 1	15	16.7	55.7	0.0056
Sample 2	15	14.0	46.7	0.0047

Following the discovery by Yang *et al.* (1958) that the tobacco and the mainstream smoke from cigarettes commonly used in the U. S. contained scopoletin (6-methoxy-7-hydroxycoumarin), these workers devised quantitative methods for its determination in cigarette tobacco and smoke. Recently, they reported quantitative results obtained for scopoletin in 24 brands of cigarettes (Yang *et al.*; in press). These findings have suggested the extension of qualitative and quantitative scopoletin studies to other commercial tobacco products. This paper reports the identification of scopoletin in all the cigars, snuff, chewing tobaccos, pipe tobaccos, pipe mixtures, and roll-your-own cigarette tobaccos analyzed. Quantitative results are reported on selected samples of each type of product as well as on the smoke from selected cigars.

## Materials and Apparatus

**Tobacco samples**—Each tobacco product tested was purchased locally on the open, retail market. Cigars studied were El Producto (Puritano), Wm. Penn (Perfecto), Robt. Burns (de Luxe), Robt. Burns (Cigarillo), White Owl (Perfecto), Roi-Tan (Perfecto), Roi-Tan (Golfer), El Verso (Bouquet), El Verso (Mellow), King Edward, Melba, Melba (Midget), Webster (Babies), Corina (Magnolia), Corina (Larks), Corina (Cigarillo), Coronitas (Perfecto), Dutch Masters (Corona de Luxe), Dutch Masters (Crown), Dutch Masters (Belvedere), Dutch Masters (President), Muriel, Royalist (New Duke), Cuesta-Ray, Antonio y Cleopatra, El Trelles, Red Dot (Perfecto), Red Dot (Cigarillo), Mississippi River, Hunter (Imperial), and

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Little Fendrich (Buds).

Pipe tobaccos and mixtures and hand-rolled cigarette tobaccos included Kite, Bugler, Revelation, Walnut, London Dock, Bond Street, Half and Half, George Washington, Edgeworth, Dill's Best, Model, Sir Walter Raleigh, Prince Albert, Velvet, Briggs, and Sutliff's Mixture No. 79. Snuffs tested were called Honest Scotch Snuff, Scotch Snuff, Sweet Mild Snuff, and Copenhagen Snuff. The chewing tobaccos studied were Bull of the Woods, Day's Work, W. N. Tinsley, Tinsley's Natural Leaf, Skoal Wintergreen Flavored, and Beech-Nut.

**Chromatography paper and chambers**—The chromatography paper used throughout these analyses has been Schleicher and Schuell No. 589, Red Ribbon. Purchased in 58x58 cm. sheets, the papers, unless otherwise noted, have been cut to a size approximately 19x58 cm. each for use. Descending chromatography in conventional 12"x24" Pyrex chromatography jars and in the standard, stainless steel interior chromatography cabinets (approximately 27½"x26"x-19½" inside dimensions) has been employed.

**Smoking machine**—To obtain cigar smoke for scopoletin analysis the cigar was smoked on a standard cigarette smoking apparatus (Phipps and Bird, Inc., Richmond, Va.) based on a design of the American Tobacco Company. The holder was modified to accommodate various cigar sizes. To trap the smoke, three Kjeldahl flasks were used in series. The first flask, 300 ml. capacity, was equipped with a spiral tube, one end of which was connected to the cigar holder and the other end of which reached the bottom of the flask. A small glass stopper was attached to the end of the side arm. During smoking, this stopper could be removed and solvent added from the side arm to wash down the smoke which condensed inside the spiral tube. Straight glass tubes were used for the second and third flasks, each of which flasks was of 100 ml. capacity.

#### Experimental

**Preparation and extraction of sample**—Each tobacco product was ground with a Wiley intermediate mill, 40 mesh screen. The samples were not dried beforehand, except for three brands of chewing tobacco, 4 CH, 5 CH, and 6 CH. These were dried at 50° for 48 hrs. prior to grinding. Approximately 4 g. of each of these ground tobacco powders were weighed and transferred into individual Soxhlet thimbles for ex-

traction.

Each 4 g. extraction was carried out in a separate Soxhlet extractor, using 250 ml. of 85 percent isopropyl alcohol for approximately 3 hrs. on a steam bath. A second extraction was made on each sample, again using 250 ml. of 85 percent isopropyl alcohol for 3 hrs. The two extracts of the 4 g. tobacco sample were combined, reduced to approximately 150 ml. in vacuo, and the volume was then adjusted with 85 percent isopropyl alcohol to 200 ml. in a volumetric flask. Aliquots of this solution were then taken for qualitative and for quantitative analyses of scopoletin.

**Smoking procedure**—Prior to smoking, 25 ml., 10 ml., and 10 ml. of an anhydrous acetone-absolute ethyl alcohol (1:1 v./v.) mixture

were placed in the first, second, and third flasks, respectively. The Kjeldahl flasks used as traps were then lowered into large evacuated flasks (modified Dewar) containing dry ice-acetone (approximately -77°) for at least 30 minutes before smoking and were kept in the cold baths throughout the smoking.

The puff duration, puff interval, and volume of smoke per puff were 3.5 seconds, 30 seconds, and 53-57 ml, respectively. Exact volume of smoke per puff, number of puffs, butt length, humidity and room temperature for the quantitative studies have been recorded in table 2.

After the cigar samples had been smoked, the trap system, still connected in series, was removed from the Dewar flasks, and was left until

Table 1—continued

(1)	(2)	(3)	(4)	(5)
Pipe and Cigarette Tobaccos				
1PC				
Sample 1	15	11.6	38.7	0.0039
Sample 2	15	10.2	34.0	0.0034
2PC				
Sample 1	15	10.0	33.3	0.0033
Sample 2	15	10.4	34.7	0.0035
3PC				
Sample 1	15	10.5	35.0	0.0035
Sample 2	15	9.4	31.3	0.0031
Chewing Tobaccos				
1CH				
Sample 1	20	5.9	14.8	0.0015
Sample 2	20	5.5	13.8	0.0014
2CH				
Sample 1	20	6.0	15.0	0.0015
Sample 2	20	6.3	15.8	0.0016
3CH				
Sample 1	20	8.7	21.8	0.0022
Sample 2	20	9.1	22.8	0.0023
4CH**				
Sample 1	20	10.0	25.0	0.0025
Sample 2	20	9.9	24.8	0.0025
5CH**				
Sample 1	20	9.0	22.5	0.0023
Sample 2	20	8.8	22.0	0.0022
Snuff				
1S				
Sample 1	20	10.4	26.0	0.0026
Sample 2	20	11.7	29.3	0.0029
2S				
Sample 1	20	12.9	32.3	0.0032
Sample 2	20	11.4	28.5	0.0029
3S				
Sample 1	20	10.0	25.0	0.0025
Sample 2	20	10.2	25.5	0.0026

\* In every case reported, 4.00 g. of tobacco product was extracted; total volume of each extract was 200 ml.

\*\* The 4.00 g. are weights after drying at 50°C for 48 hours. Brand 4CH lost 0.88% of its weight, and 5CH lost 8.23% of its weight prior to grinding.

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Table 2.—Determination of scopoletin in cigar smoke

Cigar type and brand	Weight (g.) of cigar	Volume $\pm$ 1 ml.	No. of puffs per cigar	Butt Length (mm.)	Scopoletin ( $\mu$ g) per 20 ml. aliquot	Scopoletin ( $\mu$ g) per 1 g. cigar
1C, cigarillo						
*Sample 1	11.90	54	35	30	8.0	3.4
*Sample 2	12.95	54	35	30	8.2	3.2
2C, perfecto						
**Sample 1	16.62	56	50	50	8.0	2.4
**Sample 2	16.69	56	50	50	4.5	1.4

For each sample: room temperature was 32°C; humidity, 24%; total volume of extract was 100 ml.; and 20 ml. aliquots were streaked on paper for analysis.

\*Each sample consisted of 4 cigarillos.

\*\*Each sample consisted of 2 cigars.

the temperature of the system reached that of the room. The solvent already in the three traps plus all the acetone-absolute alcohol washings were combined and transferred quantitatively into a 100 ml. volumetric flask and made to volume. Twenty milliliter aliquots of this solution of smoke condensate were used for the scopoletin analyses.

**Qualitative analyses**—For identification purposes, the scopoletin was purified by extended paper chromatography, using the basic procedure previously described (Yang, *loc. cit.*) The resulting scopoletin was checked with an authentic sample synthesized by the procedure of Aghoramurthy and Seshadri (1952). The infrared spectrum of scopoletin is shown in figure 1. The absorption spectra showed a prominent absorption maximum at 344 m $\mu$  (Yang *loc. cit.*) for the synthetic and purified samples containing sufficient scopoletin. Each scopoletin fraction from a tobacco product was also subjected to one- and two-dimensional mixed paper chromatography with authentic samples. Solvent systems used for paper chromatography were 15 percent acetic acid-water; 60 percent acetic acid-water; n-butyl alcohol-acetic acid water (6:1:2 v/v); n-butyl alcohol-benzene-pyridine-water (5:1:3:3 v/v); and nitromethane-benzene-water (2:3:5 v/v). Typical R<sub>f</sub> values for scopoletin in these solvent systems, respectively, using the S & S No. 589 paper and a temperature of 28°  $\pm$  3° were 0.47; 0.47; 0.82; 0.82 and 0.69.

**Quantitative analyses**—The quantitative determinations of scopoletin in the various tobacco products and in cigar smoke were performed by the analytical procedures already described by Yang *et al.* for scopoletin in cigarette tobacco and smoke, resp. (Yang, in press). These involved ex-

tended paper chromatography for the quantitative separation and purification of the scopoletin, elution with 50 percent ethyl alcohol-water, and spectrophotometric measurement at 344 m $\mu$ . The quantity of scopoletin present was calculated from an experimentally determined standard curve prepared from known amounts of pure, synthetic scopoletin carried through the same procedure as the tobacco or smoke scopoletin.

#### Results and Discussion

Data for the amount of scopoletin found to be present in each tobacco product containing at least 5  $\mu$ g of scopoletin per aliquot are reported in table 1. Below this concentration, the reproducibility and accuracy of the procedure become lower, and hence, for such samples only the statement is made that they contain less than 5  $\mu$ g per aliquot. Each value of column 3, table 1, is given in micrograms per aliquot and represents the average of values obtained on three aliquots. In column 4 are recorded the micrograms of scopoletin per gram of tobacco product analyzed. These values have been expressed as percentages in column 5.

All 31 brands of cigars analyzed qualitatively were found to contain some scopoletin, though apparently in different amounts. For preliminary classification purposes as to scopoletin content, the relative size of the purified scopoletin zone and the intensity of its fluorescence were used as guides, in a manner similar to that employed for the estimation of rutin in tobacco samples by Penn and Weybrew (1958). The brands were thus placed in one of the following groups: (a) relatively higher scopoletin content, nine brands; (b) medium, eight brands; (c) low scopoletin content, 12 brands; and (d)

trace amount of scopoletin, two brands. Two cigars each of six brands in group "a" which were considered likely to be highest in scopoletin content as estimated by the above method, were selected for quantitative analysis by the procedure of Yang *et al.* (in press). The quantitative results for four of these samples are recorded in table 1. The other two brands had values of less than 5  $\mu$ g per aliquot.

Analyses of the scopoletin content of five brands of regular cigarettes (Camel, Lucky Strike, Philip Morris, Chesterfield, and Old Gold) for the percentage of scopoletin in cigarette tobacco gave values in the range of 0.0066-0.012% (Yang, in press). Only 1C and 2C of the cigars tested fell in this range. These two were thus selected for quantitative determination of cigar smoke under the smoking conditions described. Cigars 1C and 2C actually carry the same brand name, but 1C is the cigarillo and 2C is the perfecto of that brand.

The smoke from 42 other cigars, including 19 other brands, were studied qualitatively for their scopoletin content. In cigar smoke there are impurities which seem to interfere even more than usual with the determination of scopoletin. And, because of the apparently very low scopoletin content of the smoke from these other cigars, quantitative studies on the smoke from cigars have been limited to 1C and 2C. The results of these analyses are shown in table 2. In the case of 2C, definite variations were found in individual cigars analyzed.

The values in table 2 indicate that 1C and 2C, by far the highest of all the cigars tested in their scopoletin content, produce a relatively lower scopoletin content in the smoke than was found in smoke from a comparable amount of tobacco in manufac-

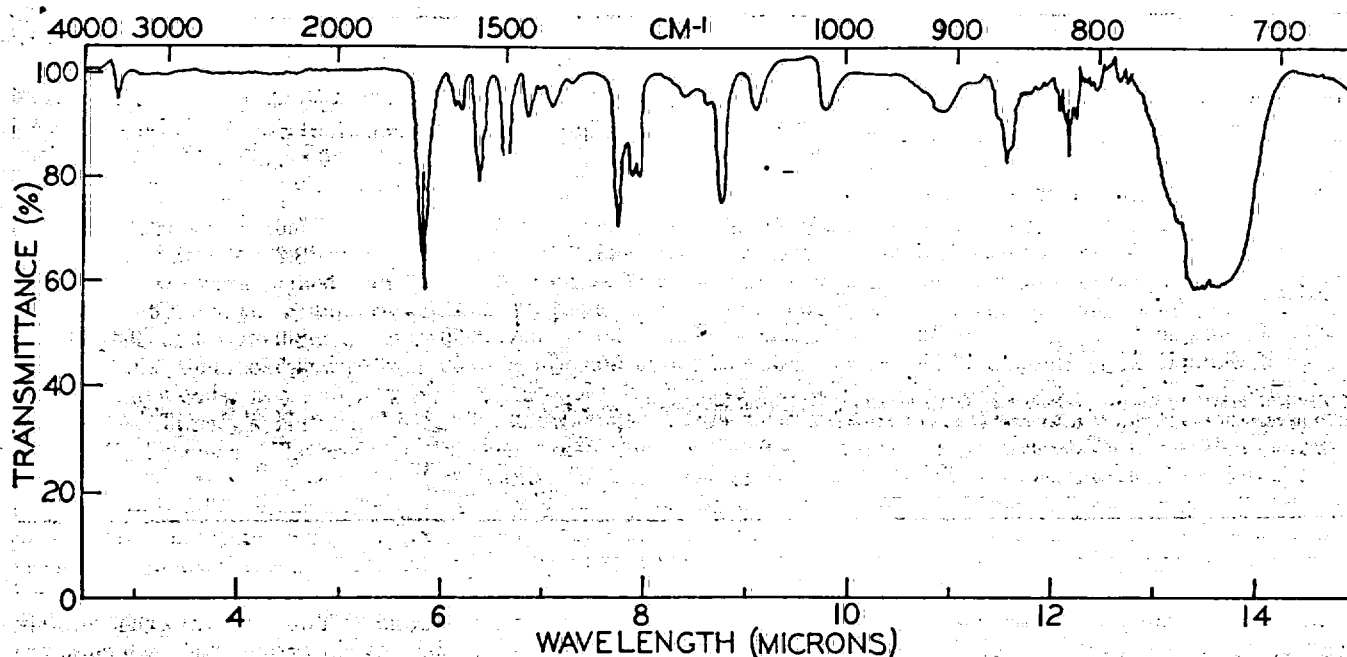


Figure 1. Infrared spectrum of scopoletin (from Perkin-Elmer Infracord).

tured cigarettes. In the latter case, the range was found to be from 10.3-27.8  $\mu\text{g}$  scopoletin per 1 g. of cigarette.

From the values shown in table 1, it is evident that both brands of hand-rolled cigarette tobacco tested contained scopoletin in an amount (column 5) that falls in the range of 0.0066-0.012% which was found for regular, manufactured cigarettes. This was also the case for the 3 brands (1PM, 2PM, and 3PM) labeled as pipe mixtures. None of these, however, approached the upper limits found for tobacco in regular cigarettes.

The scopoletin content of all the brands labeled as pipe tobacco or pipe and cigarette tobacco was slightly lower than that found in a comparable weight of the tobacco

from cigarettes.

The amount of scopoletin in the snuff and chewing tobacco samples analyzed was even lower than that of the pipe tobaccos studied. Two brands, however, (4S and 6CH) were considerably lower in scopoletin content than the other snuff and chewing tobacco samples analyzed. Both had less than 5  $\mu\text{g}$  of scopoletin per aliquot.

#### Summary

Scopoletin has been found to be present in all cigars, snuff, chewing tobaccos, pipe tobaccos, pipe mixtures, and roll-your-own cigarette tobaccos analyzed.

Quantitative results have been reported on selected samples of each type of tobacco product as well as on the smoke from selected cigars.

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